

LISTING OF CLAIMS

1. (amended) A method of determining the copy number (CN) of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase and optionally, any additional further reagents, if any, required for the amplification technique used to the sample,
- (2) performing one or more amplification cycles to amplify the nucleotide sequence NucSeqI for which the copy number has to be determined;

wherein the sample comprises contains a chromosomal chromosome-derived second nucleotide sequence II (NucSeqII), and the following amplification steps are carried out:

- (a) the first nucleotide sequence NucSeqI is amplified,
- (b) the second nucleotide sequence NucSeqII is amplified,
- (c) a third nucleotide sequence I' (NucSeqI') corresponding to the first nucleotide sequence NucSeqI and present in a control sample is amplified at various multiple dilutions, and
- (d) a fourth nucleotide sequence II' (NucSeqII') corresponding to the second nucleotide sequence NucSeqII and present in a control sample is amplified at multiple various dilutions,

wherein the ratio of the concentration[[s]] of nucleotide sequence NucSeqI' and the concentration of NucSeqII' is known,

wherein the amplification[s] of the third and fourth nucleotide sequences NucSeqI' and NucSeqII' at multiple various dilutions results in the generation of allows standard curves SC_i, SC_I and SC_{II}, respectively with i being I or II to be made, such that the concentrations of NucSeqI and NucSeqII are determined by using the respective standard curves SC_I and SC_{II} SC_i, such that the relative concentrations allows the relative copy number CN of sequence NucSeqI relative to (versus nucleotide sequence II) NucSeqII is to be determined using the formula

$$CN = \frac{\underline{Conc-I}_{SC_I} \quad [H]_{SC_I}}{\underline{Conc-II}_{SC_{II}} \quad [H]_{SC_{II}}}$$

wherein

- (i) CN is the relative copy number of NucSeqI relative to over NucSeqII) in the sample;
- (ii) Conc-I_{SC_I} is the concentration of NucSeqI determined using standard curve SC_I; and

{H_{SCII}} (iii) Conc-II_{SCII} is the concentration of NucSeqII determined using standard curve SC_{II}, and

wherein:

at least one pair of amplification reactions selected from chosen from i) (a) and (b), and [[ii]] (c) and (d) is performed in a single container and monitored spectrophotometrically during amplification, and

~~the third nucleotide sequence NucSeqI' and fourth nucleotide sequence NucSeqII' are localized~~ reside on a single vector.

2. (amended) A method according to claim 1, wherein characterized in that the an absolute copy number is determined by multiplying ~~the copy number~~ CN by the absolute ~~copy~~ number of copies of sequence NucSeqII per cell.

3. (amended) A method according to claim 1 or 2, wherein characterized in that at least two and also more different third nucleotide sequences NucSeqI' sequences used for measuring a corresponding number of different first nucleotide sequences NucSeqI sequences are localized reside on a single vector.

4. (amended) A method according to claim 1 any of the preceding claims, characterized in that wherein the sequences of the first nucleotide sequence NucSeqI is the same as and the third nucleotide sequence NucSeqI' are the same.

5. (amended) A method according to claim 1 any of the preceding claims characterized in that wherein the sequences of the second nucleotide sequence NucSeqII is the same as and the fourth nucleotide sequence NucSeqII' are the same.

6. (new) A method according to claim 2, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

7. (new) A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. (new) A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. (new) A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. (new) A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(new)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(new)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(new)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(new)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(new)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(new)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.